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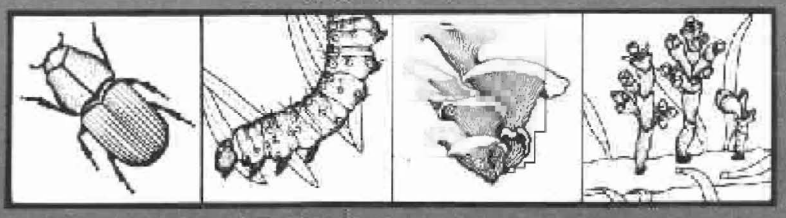

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PATHOGENIC *FUSARIUM* ON SPRUCE SEED FROM THE TOWNER NURSERY, NORTH DAKOTA

by

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ABSTRACT

Five seedlots of Colorado blue spruce and three seedlots of Black Hills spruce were sampled for *Fusarium* contamination. All seedlots contained some seed and/or debris with *Fusarium*. Levels of contamination were greatly reduced by treating seed with running water rinses for 48 hours or with chemical sterilants such as sodium hypochlorite (bleach), hydrogen peroxide, captan, and benomyl. All three species of *Fusarium* commonly isolated from seed, *F. oxysporum*, *F. solani*, and *F. tricinctum*, were pathogenic to blue spruce germlings after test tube inoculations. Reducing the amount of seed contamination will help reduce future losses from pre- and post-emergence damping-off.

INTRODUCTION

Seeds of many different conifer species are often contaminated with *Fusarium* or other pathogens which may cause pre- or post-emergence diseases (James 1985). If poor germination of certain seedlots or higher than normal damping-off occurs, seed-borne pathogens may be involved. To adequately assess the importance of seed-borne pathogens, suspected seedlots should be periodically screened for pathogen contamination.

Growers at the North Dakota Forest Service Nursery in Towner were concerned that certain Colorado blue spruce (*Picea pungens* Engelm.) and Black Hills spruce (*Picea glauca* var. *albertiana* (S. Brown) Sarg.) seedlots were experiencing unusually high disease losses. They thought that seed-borne *Fusarium* spp. might be at least partially responsible for these losses. Therefore, investigations were conducted to quantify relative levels of *Fusarium* contamination within selected seedlots. Several *Fusarium* isolates obtained from seed were also screened for their ability to incite disease on young spruce germlings.

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MATERIALS AND METHODS

Five blue spruce and three Black Hills spruce seedlots (table 1) were sampled for contamination by Fusarium and other potentially pathogenic fungi. Six of these seedlots, three Black Hills spruce and three blue spruce, were collected from counties in North Dakota between 1981 and 1984. Two of the blue spruce seedlots were collected in Colorado 6 years ago.

Table 1.--Spruce seedlots sampled for Fusarium spp. from the Towner Nursery, North Dakota.

Seedlot	Year collected	Species[1]	Location collected
1-81	1981	CBS	Bottineau & McHenry Co., ND
1-83	1983	BHS	Towner, ND
1-84	1984	BHS	Towner, ND
2-84	1984	CBS	Bottineau & Molberg, Co., ND
3-84	1984	CBS	Pembina Co., ND
79155A	1979	CBS	San Juan NF, CO
79155B	1979	CBS	San Juan NF, CO
McHenry	1981	BHS	Bottineau & McHenry Co., ND

[1]CBS = Colorado blue spruce (Picea pungens Engelm.)
BHS = Black Hills spruce (Picea glauca var. albertiana (S. Brown) Sarg.)

Seeds were treated as outlined in table 2. Debris accompanying seed, such as small rocks, pieces of wood, and aggregations of resin, were also screened for Fusarium contamination. The medium on which seed were incubated following eight treatments was selective for Fusarium spp. (Komada 1975). Following two of the treatments (nos. 4 & 6), seed were incubated on malt agar, a general non-selective medium, to ascertain presence of other fungi. Following incubation at 21°C under 12-hour diurnal cycles of cool fluorescent light for 5-7 days, emerging fungi were transferred to potato dextrose agar (PDA) and identified. Major keys for identification of Fusaria included those by Booth (1971) and Gerlach and Nirenberg (1982).

Six Fusarium isolates (three species) prepared from spruce seed were selected for germling pathogenicity tests. Germlings were obtained by germinating surface sterilized (10 percent bleach for 5 minutes and rinsed in sterile distilled water) blue spruce seed in small Leach® cells filled with peat-vermiculite growing medium and covered with perlite. Germinating seed were kept moist in a growth chamber at about 24°C under 12-hour diurnal cycles of cool fluorescent light. Germlings were 4-6 weeks old when inoculated.

Table 2.--Treatments to determine relative occurrence of Fusarium spp. on spruce seed from the Towner Nursery, North Dakota.

Treatment No.	Treatment
1.	Seed placed directly on selective medium.[1]
2.	Debris (rocks, wood pieces, resin aggregations) placed directly on selective medium.
3.	Seed soaked in standing tap water for 24 hours and placed on selective medium.
4.	Seed soaked in standing tap water for 24 hours and placed on malt agar.
5.	Seed rinsed under running tap water for 48 hours and placed on selective medium.
6.	Seed rinsed under running tap water for 48 hours and placed on malt agar.
7.	Seed soaked in 10 percent bleach (0.525 percent sodium hypochlorite) for 2 hours and placed on selective medium.
8.	Seed soaked in 3 percent hydrogen peroxide for 2 hours and placed on selective medium.
9.	Seed soaked in standing tap water for 24 hours and dusted with Captan.[2]
10.	Seed soaked in standing tap water for 24 hours and dusted with benomyl.[3]

[1]Selective medium for Fusarium spp. (Komada 1975).

[2]N-(Trichloromethylthio)-4-cyclohexene-1,2-dicarboximide.

[3]Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate.

Pathogenicity tests were conducted within sterile test tubes containing two pieces of inverted filter paper moistened with 6 ml distilled water. Each test tube contained a 1 cm piece of agar inoculum from 7 day old cultures grown on PDA. Pieces of agar without the fungi served as controls. Germlings were carefully removed from cells, washed thoroughly under running tap water, and placed with their roots in direct contact with the inoculum.

After incubation, at about 21°C under cool fluorescent light for 14 days, inoculated seedlings were assayed for fungal infection. The entire root and hypocotyl of each germling was aseptically cut into 1-1.5 cm pieces and placed on the Fusarium-selective medium to recover the inoculated fungus. Plates

were incubated at 21°C for 5-7 days, after which extent of Fusarium colonization was measured. Percentage of germlings infected, number of root-hypocotyl pieces infected, and percentage of root-hypocotyl length colonized were calculated.

RESULTS

Some seed and/or debris from each sampled seedlot were contaminated with Fusarium spp. (table 3). Extent of contamination was particularly high in three seedlots (1-84, 1-81, and 3-84). Also, debris accompanying seed was heavily contaminated with Fusarium in five seedlots.

Table 3.--Occurrence of Fusarium spp. on spruce seed from Towner Nursery, North Dakota.[1]

	Treatment[3]							
Seedlot[2]	1	2	3	5	7	8	9	10
1-81	42.0	36.8	14.0	0	0	0	0	0
1-83	26.0	20.7	10.0	0	0	2.0	0	0
1-84	78.0	59.1	50.0	4.0	0	0	0	0
2-84	0	12.5	0	2.0	0	0	0	0
3-84	52.0	40.0	4.0	0	2.0	2.0	0	0
79155A	4.0	32.0	0	0	0	0	0	0
79155B	8.0	0	6.0	0	0	0	0	0
McHenry	18.0	0	0	0	0	0	0	0
All lots	28.5	18.2	10.5	0.8	0.3	0	0	0

[1] Figures in table are percent of sampled seed colonized by Fusarium spp.

[2] See table 1 for seedlot descriptions.

[3] See table 2 for treatment descriptions. Treatments 4 and 6 are excluded because they involved placing seed on malt agar; Fusarium spp. were often masked by faster growing fungi on this medium.

Soaking seed in standing water for 24 hours, a common pre-germination practice at many conifer seedling nurseries, only reduced Fusarium contamination slightly. Levels of fungal colonization of seed were still high in several seedlots. Rinsing seed for 48 hours under running tap water eliminated

Fusarium contamination in all but two seedlots. The chemical sterilizing treatments were about equally effective in reducing Fusarium contamination. Although some of these chemicals would be expected to influence seed germination, their effects on germination were not monitored.

Three species of Fusarium were consistently isolated from spruce seed. Detailed descriptions of these fungi are included in the Appendix. The most frequently isolated species was F. oxysporum Schlect. At least two morphologically distinct strains of this species were consistently observed. One produced deep violet pigment in the culture medium and the other a bright orange aerial mycelium and very faint pigment. The other species isolated were F. solani (Mart.) Sacc., and F. tricinctum (Corda) Sacc. Other organisms isolated from spruce seed (treatments 4 and 6 - table 2) included Rhizopus, Mucor, Alternaria, Trichoderma, Aureobasidium, Penicillium, Phoma, and several unidentified species of bacteria. Pathogenicity of these non-Fusarium organisms was not tested.

All three Fusarium species were pathogenic to blue spruce germlings (table 4). Some differences in virulence, as manifested by extent of hypocotryl-root colonization, were evident among the tested isolates. Two of the F. oxysporum isolates (85-23 and 85-26) were about equally virulent and generally more aggressive than the third isolate, (85-19). The two F. tricinctum isolates tested varied greatly in virulence. Isolate 85-36 was very aggressive, whereas isolate 85-25A was consistently less virulent. The F. solani isolate was as virulent as several of the other Fusarium isolates tested. Differences in virulence were apparently not related to morphological differences of the tested isolates and could only be determined from pathogenicity tests.

DISCUSSION

These studies indicated that five of eight spruce seedlots from the Towner Nursery were heavily contaminated with at least three species of Fusarium. All three species were pathogenic to spruce germlings and were probably responsible for both pre- and post-emergence damping-off losses at the nursery. Unfortunately, such high levels of seed contamination are not uncommon for conifer species, particularly if seed are collected from cones that have been in contact with the ground for extended periods of time, such as in squirrel caches.

The other fungi isolated from seed are common inhabitants of conifer seed (James and Genz 1982). Most were probably not as pathogenic on spruce germlings as Fusarium, although tests of their pathogenicity were not conducted.

These studies also showed that several low-cost treatments effectively reduced seed contamination to acceptable levels. The running water rinse was very effective and, based on previous experience (James 1983b), probably would not affect seed germination as much as some of the chemical sterilants. However, effects of chemical treatment effects on spruce seed germination should probably be quantified.

Past experience with seedborne Fusaria indicate that F. oxysporum and F. solani are common on conifer seed (Graham and Linderman 1983; James 1983a; James 1984). However, occurrence of F. tricinctum on conifer seed has not been previously reported, although it could have been grouped into the "roseum"

complex by investigators. This soilborne species may be an aggressive pathogen on wheat seedlings and also causes diseases of carnations, maize, lupine, and apples (Seemuller 1968). Its importance as a pathogen of conifer seedlings is unknown.

Table 4.--Infection of Colorado blue spruce germlings by Fusarium isolates obtained from spruce seed from Towner Nursery, North Dakota.

Isolate	Fusarium species	% germlings infected	% pieces infected[1]	Average percent germling colonization[2]
85-19	F. oxysporum	100.0	84.4	68.8
85-23	F. oxysporum	100.0	87.6	83.9
85-26	F. oxysporum	100.0	91.9	89.4
85-22	F. solani	100.0	83.9	81.3
85-25A	F. tricinctum	90.0	54.5	48.3
85-36	F. tricinctum	100.0	95.3	92.4

[1]Percent of main stem pieces of germlings colonized by inoculated isolate.

[2]Percent of the linear distance of main stem of germlings colonized by inoculated isolate.

As a result of these studies, it is recommended that growers at the Towner Nursery operationally treat spruce seed with either a running water rinse or chemical sterilants prior to sowing to reduce levels of Fusarium contamination and subsequent disease losses. Such treatments should result in improved seed germination and denser stands of seedlings.

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APPENDIX

Descriptions of Fusarium spp. isolated from spruce seed from the Towner Nursery, North Dakota.

Fusarium oxysporum Schlect. Isolates 85-19, 85-23, 85-26.

- colonies mostly fast growing, reaching 6.5 to 8.0 cm diameter in 8 days at 25° C on PDA.
- two morphologically distinct colonies were evident:

  85-23: Abundant, fluffy white aerial mycelium with a deep violet pigment that penetrates the agar surface.

  85-19 and 85-26: Abundant fluffy orange aerial mycelium with a very slight violet pigmentation produced under the colony.

- light orange-colored sporodochia formed abundantly on carnation leaf agar.

- microconidia abundant, 1- to 2-celled cylindric to ellipsoid, oval, often slightly curved and borne on short, unbranched phialides.

- macroconidia abundantly produced within sporodochia, falcate, equally and gradually tapering toward both ends, with a pointed apical cell and a distinctly pedicellate basal cell.

- chlamydospores not abundantly formed in young cultures, but a few are formed as cultures age.

Fusarium solani (Mart.) Sacc. Isolate 85-22

- colonies mostly fast growing, reaching 7.5-8.0 cm in 8 days at 25°C on PDA.

- colonies with mostly appressed, moist, somewhat orange mycelium.

- no distinct pigment produced.

- abundant cream-colored pionnotes formed over the surface of the colony.

- abundant white- cream-colored sporodochia formed on carnation leaf agar after 12 days.

- microconidia abundantly produced in pionnotes, 1- to 2-celled, oval, ellipsoid to subcylindric, borne on long irregularly branched conidiophores.

- macroconidia abundantly produced within sporodochia, rather thick-walled, with a short and blunt apical and an indistinctly pedicellate basal cell, and mostly 3-5 septate.

- chlamydospores abundantly produced, intercalary or terminal, and mostly globose.

Fusarium tricinctum (Corda) Sacc. Isolates 85-25A, 85-36.

- colonies moderately fast growing, reaching 5.0-6.0 cm diameter in 10 days at 25°C on PDA.

- aerial mycelium abundant, densly cottony, giving the culture a cushion-like appearance, margin mostly irregularly lobed. Mycelium white to pink in color.

- pigmentation intensely carmine, particularly at the agar surface.

- sporulation (microconidia) abundant, giving the culture a powdery appearance. Sporodochia abundant (isolate 85-25A) or sparse (isolate 85-35) on carnation leaf agar; orange to cream colored.

- microconidia abundant, mostly 1 celled, lemon shaped (napiform, pyriform, or citriform) produced on short, mostly unbranched phialides.

- macroconidia slender, falcate, widest in the center and tapering evenly to each end, 3-5 septate with a distinct pedicellate basal cell.

- chlamydospores not common in young cultures, but well formed as intercalary chains in hyphae.